

Pretreatment and Enzymatic Hydrolysis of Sorghum Bran¹

D. Y. Corredor,² S. Bean,³ and D. Wang^{2,4}

ABSTRACT

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Sorghum bran has potential to serve as a low-cost feedstock for production of fuel ethanol. Sorghum bran from a decortication process (10%) was used for this study. The approximate chemical composition of sorghum bran was 30% starch, 18% hemicellulose, 11% cellulose, 11% protein, 10% crude fat, and 3% ash. The objective of this research was to evaluate the effectiveness of selected pretreatment methods such as hot water, starch degradation, dilute acid hydrolysis, and combination of those methods on enzymatic hydrolysis of sorghum bran. Methods for pretreatment and enzymatic hydrolysis of sorghum bran involved hot

water treatment (10% solid, w/v) at 130°C for 20 min, acid hydrolysis (H₂SO₄), starch degradation, and enzymatic hydrolysis (60 hr, 50°C, 0.9%, v/v) with commercial cellulase and hemicellulose enzymes. Total sugar yield by using enzymatic hydrolysis alone was 9%, obtained from 60 hr of enzyme hydrolysis. Hot water treatment facilitated and increased access of the enzymes to hemicellulose and cellulose, improving total sugar yield up to 34%. Using a combination of starch degradation, optimum hot water treatment, and optimum enzymatic hydrolysis resulted in maximum total sugar yield of up to 75%.

A dramatic increase in ethanol production using the current grain starch-based technology may have resource limitations because grain production for ethanol will compete for the limited agricultural land needed for food and feed production (Sun and Cheng 2001). The United States needs more than 140 billion gallons (530 billion liters) of fuels per year for automobiles alone (U.S. Department of Transportation 2006). Using 100% of the 2005 corn crop (10.35 billion bushels or 260 million tons) for ethanol production would only produce 27.9 billion gallons (105 billion liters), which only meets ≈14–16% of our demands (Hamelinck et al 2005). It is obvious that other feedstocks such as agricultural residues, wood, municipal solid wastes, and wastes from pulp and paper industry are potential resources for low-cost ethanol production (Mielenz 2001). These types of “biomass” consist of primarily cellulose (35–50%), hemicellulose (20–35%), and lignin (10–25%) (Saha and Bothast 1997). It is estimated that ≈50 billion gallons (190 billion liters) of ethanol could be produced from current biomass wastes in the United States (Saha 2004).

Bran from sorghum processing such as decortication can be classified as cellulosic material, and it represents a renewable resource that can be used as a potential feedstock for ethanol fermentation because of its high carbohydrate content. In this study, the sorghum bran is a mixture of sorghum pericarp, some germ tissue, and residual starch from the endosperm extracted during the decortication process. Sorghum structural carbohydrates, or cell-wall polysaccharides, primarily consist of hemicellulose and cellulose (Bailey 1973).

Decortication is a process to remove the hull or outer layer of the grains for production of high-quality food products or other special purposes. The removed bran is considered a low value by-product. With 10% decortication and 56 lb (25.4 kg)/bu, ≈5.6 lb (2.5 kg) of bran can be obtained from each bushel of sorghum, which could then be converted to ≈3.19 lb (1.45 kg) of fermentable sugars. These fermentable sugars can theoretically yield ≈0.27 gal (1.02 L) of ethanol.

The general idea for degradation of lignocellulosic materials is combining pretreatment procedures with enzyme hydrolysis (Saha 2003). Pretreatment methods have been developed to enhance the hydrolysis of cellulosic materials by enzymes (Saha and Bothast 1999; Abbas et al 2004; Dien et al 2004; Palmarola et al 2004; Varga et al 2004; Saha et al 2005). However, there are no reported approaches to converting sorghum bran to value-added products, especially to fermentable sugar.

Dilute acid pretreatment (H₂SO₄ or HCl) of native lignocellulose greatly increases the conversion rate of cellulosic biomass and takes away hemicellulose, either in combination with the breakdown of cellulose to glucose or before acid hydrolysis of cellulose (Chung et al 2005). It also hydrolyzes some hemicellulose components to syrup of monomeric sugars, exposes cellulose to enzymatic digestion, and solubilizes heavy metals that may be contaminating feedstocks (Ingram et al 1997; Lavarack et al 2002).

Hot water (HW) (120–200°C, 5–30 min) and supercritical water treatments may also be suitable methods for sorghum bran, even though there is limited information available (Saha and Bothast 1997; Miyafuji et al 2005). The major advantage of HW is that there is no addition of chemicals during pretreatment (Van Walsum et al 1996). Hot water offers the potential advantage of high pentose recovery compared with steam explosion, and less corrosive operating conditions, as with dilute acid pretreatment; it also prevents further degradation of monosaccharides and yields little waste production (Van Walsum et al 1996).

The greatest potential for ethanol production from biomass also lies in enzymatic hydrolysis of cellulose and hemicellulose using cellulase and hemicellulase enzymes (Saha and Bothast 1997; Saha 2003; Knauf and Moniruzzaman 2004). Although the structure of hemicellulose is more complex than cellulose and requires several different specificities for complete hydrolysis; the polysaccharide does not form tightly packed crystalline structures like cellulose does and thus is more accessible to enzymatic hydrolysis (Saha 2003). However, preliminary studies on corn bran demonstrated that there are no suitable commercial hemicellulase preparations that can hydrolyze corn bran hemicellulose to monomeric sugars efficiently (Saha and Bothast 1999). In cellulose, enzymatic hydrolysis requires mild conditions and long periods of time (Saha 2004). Maximum cellulase and β-glucosidase activities occur at 40–60°C, pH 4.0–5.0, 24–72 hr, and 0.3–1% enzyme (Saha 2004) but optimal conditions may change with the hydrolysis residence time (Taherzadeh and Niklasson 2004).

Finally, greater lignin content blocks enzyme accessibility, adsorbs enzymes nonproductively, causes end-product inhibition, and reduces rate and yield (Knauf and Moniruzzaman 2004). In addition to lignin, cellobiose and glucose also act as strong inhibitors of cellulases (Knauf and Moniruzzaman 2004). The presence

¹ Contribution No. 06-361-J from Kansas State Agricultural Experiment Station, Manhattan, KS 66506.

² Department of Biological and Agricultural Engineering, Kansas State University, Manhattan, KS 66506.

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⁴ Corresponding author. Phone: 785-532-2919. Fax: 785-532-5825. E-mail: dwang@ksu.edu

of an efficient β -glucosidase enzyme system is essential because it converts the cellobiose to glucose and liberates cellulases from inhibition by cellobiose, resulting in significant improvements in hydrolysis of cellulosic materials (Kadar et al 2004).

Combining pretreatments such as high temperatures with dilute acid could increase the efficiency of hydrolysis of cellulosic materials (Taherzadeh and Niklasson 2004). Although there are several methods available for biomass pretreatment, in general, the selectivity of methods is highly restricted by the nature of the raw materials. This research aimed to evaluate the effectiveness of selected pretreatment methods such as hot water, starch degradation, dilute acid hydrolysis, and the combination of those methods on enzymatic hydrolysis of sorghum bran into fermentable sugars.

MATERIALS AND METHODS

Materials

Sorghum bran ($\approx 8\%$ moisture content) was obtained from 10% decortication processing of commercial sorghum grain. Total time of decortication process was 4 min. A tangential abrasive dehulling device (TADD) equipped with an 80-grit abrasive pad was used for sorghum decortication (Venebles Machine Works, Canada). The abrasive pad was shimmed to minimum distance from the upper plate. Sorghum bran removed during decortication had a particle size < 2 mm and was collected for analysis and pretreatment. The moisture content of these samples was determined by using Approved Methods 44-15A (AACC International 2000). The sorghum bran was refrigerated and stored. Various commercial enzyme samples were obtained from different manufacturers: Cell Wall Degrading Complex (Viscozyme L-V2010) (hemicellulase, cellulase, arabinase, and xylanase complex), Novozyme 188 (β -glucosidase) (66.8×10^3 IU/mL) from Novozyme (Franklinton, NC), and GC 880 (β -glucanase and xylanase complex) from Genencor Int. (Rochester, NY). Sugars were purchased from Fischer Scientific.

Hot Water Treatment (HW)

The HW treatment was performed in a pressure reaction apparatus (model 4525 of Parr Instrument Company, Moline, IL). A central composite design with five levels of time (5, 10, 20, 30, and 34 min) and temperature (111, 120, 140, 160, and 168°C) was used in original sorghum bran to identify optimum processing conditions.

Sorghum bran was mixed with distilled water to obtain 10% dry matter. The slurry was loaded into a 1-L reactor and treated according to the central composite design. After treatment, the slurry was removed from the reactor and the residual sorghum bran separated by centrifugation at $3,760 \times g$ for 10 min at room temperature. The resulting sorghum bran cake was dried at 49°C for 24 hr. This was then analyzed for its chemical composition and susceptibility to further enzymatic hydrolysis. The liquid was collected for sugar analysis.

Starch Degradation (SD)

Termamyl 120 L (0.01 mL of α -amylase/g of dry starch) (Novozymes North America, Franklinton, NC) was used for starch liquefaction. A 20-L steam jacket kettle (model TDC/2-10, Dover Corporation), with 10 L of medium containing 20% sorghum bran (DM) and 1.5 mL of Termamyl 120 L, was heated (95°C) with agitation (140 rpm) (mixer model 750-0230, Barnant, Barrington, IL) for 45 min at pH 5.8. After decreasing the temperature to 80°C, more Termamyl 120 L (2.5 mL) was added and liquefaction allowed to proceed a further 30 min with continuous agitation at 140 rpm. Amyloglucosidase solution (3,000 U/mL) was used for starch saccharification, based on 150 U/g of dry starch at 60°C, with continuous agitation at 140 rpm for 30 min. After saccharification, the residual sorghum bran was centrifuged at $3,760 \times g$ at room temperature for 10 min (programmable centrifuge model IEC

PR-7000M, International Equipment Company, Needham Heights, MA.). Sorghum bran cake was dried at 49°C for 24 hr and collected for chemical analysis. Liquid was collected for sugar analysis.

Enzymatic Hydrolysis (EH)

Original sorghum bran was mixed with water to form bran slurry with 10% solid content and treated with a mixture of enzymes containing multifect GC 880, cell-wall-degrading complex, and Novozyme 188 at the dose level of 0.33%, v/v, for each one. The enzyme hydrolysis was preliminary conducted in flasks with 100 mL of slurry at 50°C for 48, 60, and 72 hr in a water bath shaker with agitation speed of 140 rpm, respectively. The unhydrolyzed sorghum bran was separated by centrifuging at $3,760 \times g$ for 10 min at room temperature. Liquid was collected for sugar analysis. The sediment was dried at 49°C for 24 hr and collected for chemical analysis.

Hot Water Treatment Followed by Enzymatic Hydrolysis (HW-EH)

Sorghum bran was exposed to HW and then treated by EH. The remaining sorghum bran was separated by centrifugation at $3,760 \times g$ for 10 min at room temperature. Liquid was collected for sugar analysis. The sediment was dried at 49°C for 24 hr and collected for chemical analysis.

Starch Degradation, Hot Water, and Acid Hydrolysis (SD-HW-AH)

Sorghum bran slurry (20% dm) was treated with enzymes for SD. After centrifugation, the remaining solid (10% dm) was mixed with 3% (w/w) H_2SO_4 (g of H_2SO_4 /g of dry sorghum bran) before HW treatment at 130°C for 20 min. The remaining solid after HW was separated by centrifugation at $3,760 \times g$ for 10 min at room temperature. Liquid was collected for sugar analysis. The sediment was dried at 49°C for 24 hr and collected for chemical analysis.

Starch Degradation, Hot Water, and Enzymatic Hydrolysis (SD-HW-EH)

Sorghum bran slurry (20% dm) was treated with enzymes for SD. After centrifugation, HW was applied at 130°C for 20 min to the remaining solid. The treated sorghum bran was adjusted to pH 5.0 with NaOH or HCl before enzymatic hydrolysis. Optimization of EH was done after starch degradation, according to a central composite design with five levels of time (2, 12, 36, 60, and 70 hr) and percentages of enzymes (0.03, 0.2, 0.6, 1, and 1.2% of each enzyme) at 50°C. The unhydrolyzed sorghum bran was separated by centrifugation at $3,760 \times g$ for 10 min at room temperature. Liquid was collected for sugar analysis. The sediment was dried at 49°C for 24 hr and collected for chemical analysis.

Analysis Methods

Starch content was determined by using commercially available kits from Megazyme (Bray, Ireland), according to Approved Method 76-13 (AACC International 2000). Protein was determined by nitrogen combustion using a nitrogen determinator (Leco FP-528, St. Joseph, MI), according to Approved Method 46-30 (AACC International 2000). Nitrogen values were converted to protein content by multiplying by 6.25. Crude fiber, fat, and ash were determined by AOAC standard methods (AOAC, 1995). Neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and crude fiber (CF) were determined by ANKOM technology methods. NDF measures all the fibers including hemicellulose, cellulose, and lignin. ADF measures cellulose and lignin content in the fiber. Hemicellulose was estimated by the difference between NDF and ADF. Cellulose was estimated by the difference between ADF and ADL.

Concentrations of sugars were determined by HPLC using an RCM-monosaccharide column (300×7.8 mm) (Bio-Rad, Richmond, CA) and refractive index detector. Samples were run at

85°C and eluted at 0.6 mL/min with H₂O. Hexose yield was counted as the final amount of glucose derived from starch and cellulose. Pentose yield was counted as the final amount of pentose sugars derived from hemicellulose.

Analysis of variance (ANOVA) and least significant difference (LSD) were determined using statistical software (SAS Institute, Cary, NC). The central composite design approach (Myers and Montgomery 1995) was used to study the optimal variables, including temperature and time for hot water treatment. The central composite design is a type of response surface methodology (RSM). RSM is a general linear model in which attention is focused on characteristics of the fit response function, in particular, where optimum response values occur. The pretreatment yield data were analyzed for model fit using the RSM software (Design Expert 5.0, Stat-Easy).

RESULTS AND DISCUSSION

Chemical Composition of Sorghum Bran

The sorghum bran contained 30% starch, 18% hemicellulose, 11% cellulose, and <1% acid detergent lignin (ADL) (Table I). Overall, the total carbohydrate composition was 60% while protein amounted for ≈10%. All the components measured accounted to 91.1% of the dried material. The residual material (not tested) included extractables or minerals. Corn bran is composed of ≈11–23% starch, 33–35% hemicellulose, 12–18% cellulose, 8–10% lignin, 11–12% protein, 2–3% oil, and 6% ash (Abbas et al 2004; Dien et al 2004). Compared with corn bran, the sorghum bran contained significantly more starch, less lignin, and less hemicellulose and cellulose, probably as a result of differences in processing techniques or due to differences in grain composition between maize and sorghum. As we explained above, the decortication process removes the outer layers of sorghum, mainly pericarp, but it includes some germ tissue and residual starch from the endosperm. The starch content (30%) was probably because of the small portion of endosperm extracted during decortication process. The lower lignin content of sorghum bran suggests that the lignin-polysaccharide matrix plays a less relevant role as inhibitor of the enzymatic hydrolysis than it does in corn bran (Knauf and Moniruzzaman 2004).

Effect of Hot Water Treatment on Sorghum Bran

Results of treatment with HW were analyzed by using RSM. The recovery of hemicellulose and cellulose after treatment was used to calculate the efficiency of the treatment. Linear and quadratic models were developed to study the effects of temperature and time on fermentable sugar yields on both cellulose and hemicellulose.

Figures 1 and 2 show a three-dimensional display of the response surface for the yield of cellulose and hemicellulose after HW treatment, respectively. Maximum cellulose yield (22.4%) was reached at 140.5°C and a resident time of 20.3 min (Fig. 1),

whereas the maximum hemicellulose yield (26.6%) was reached at 120°C and resident time of 10 min (Fig. 2). The desirability function was used to maximize the combination of the two responses. The optimal condition was found at 130°C and 20 min, with a desirability function of 0.67. The following results related to HW treatment were based on the optimal conditions (130°C and 20 min).

Table II shows chemical composition of bran after HW treatment compared with the original sample. Final hemicellulose and cellulose contents in the treated solids were 21.8 and 21.3%, respectively. Concentration of starch decreased 58% and concentration of hemicellulose and cellulose increased 24% and >100%, respectively. Starch is well known to be insoluble in water; however, with continued heating, the starch becomes distorted and soluble starch is released into the solution, this solubilization is

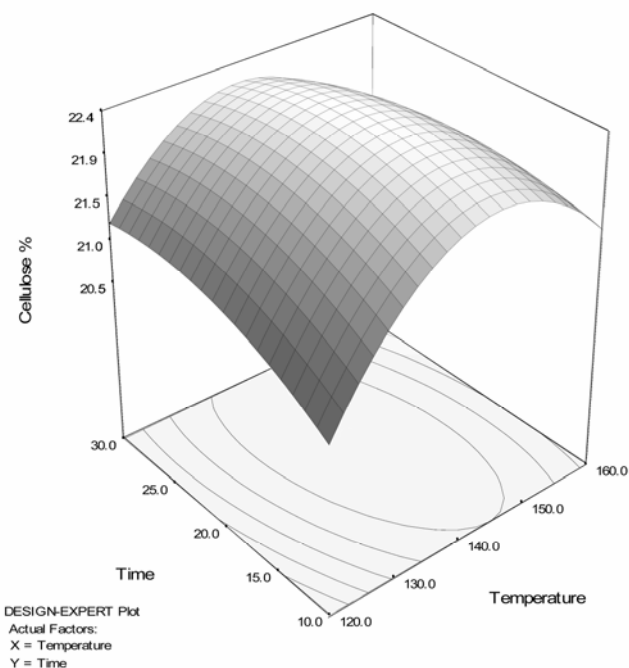


Fig. 1. Response surface of cellulose yield after hot water treatment.

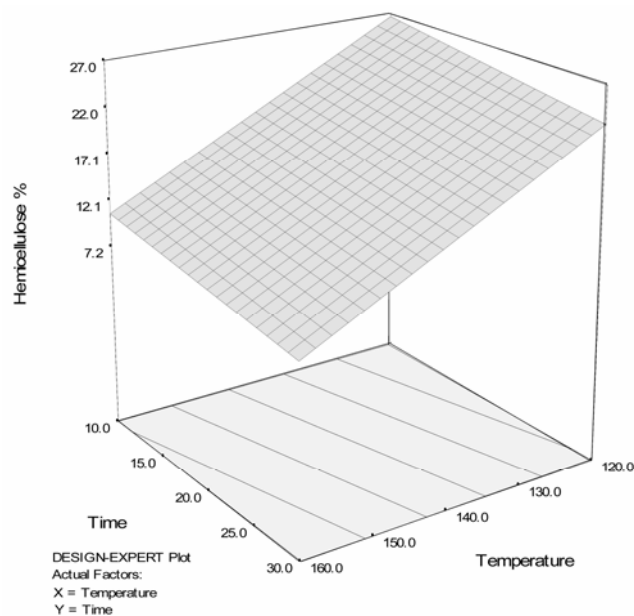


Fig. 2. Response surface of hemicellulose yield after hot water treatment.

TABLE I
Chemical Composition of Original Sorghum Bran^a

Component	Sorghum Bran (% db)
Carbohydrates	
Starch	29.7 ± 0.64
Cellulose	10.9 ± 0.1
Hemicellulose	17.5 ± 0.85
Acid detergent lignin	0.7 ± 0.1
Crude fat	8.3 ± 0.4
Crude fiber	9.3 ± 0.3
Crude protein	10.3 ± 0.07
Ash	2.7 ± 0.02
Total	91%

^a Mean values of four replicates.

continuous and would be at a temperature in excess of 120°C (Hoseney 1994). This could be one of the reasons of increment in concentration of cellulose and hemicellulose. The result indicates that HW treatment dramatically increases exposure of cellulose and hemicellulose and also helps to fractionate them.

Starch Degradation

Because the initial amount of starch was $\approx 30\%$, a starch degradation process was studied as a pretreatment to remove starch before enzymatic hydrolysis of hemicellulose and cellulose fractions. Table II shows the chemical composition of bran after starch degradation compared with the composition of the original bran. After SD, percentage of starch decreased 83%, whereas hemicellulose and cellulose percentages increased 27 and 72%, respectively. The hexose yield was 0.28 g of glucose/g of original bran, corresponding to 85% of the theoretical yield (Fig. 5). Starch degradation allows concentrating the remaining components and gives a final solid rich in hemicellulose and cellulose.

Enzymatic Hydrolysis of Sorghum Bran

Enzymatic hydrolysis was conducted to compare sugar yield before and after pretreatments. Table III shows the recovery and yield of sugars after EH. Maximum recovery of sugars was obtained with hydrolysis time of 72 hr for pentoses, 48 hr for hexoses, and 48 hr for total sugar yield. Maximum total sugar yields were 0.03 g of hexose/g of fiber and <0.01 g of pentose/g of fiber, which corresponds to a total sugar yield of 11%. Enzymatic hydrolysis ending at 60 and 72 hr only recovered 8 and 6% of the theoretical sugars, respectively. This percentage is calculated only on the basis of sugars recovered from cellulose and hemicellulose because the used enzymes cannot degrade starch. This result demonstrates that 1) enzymatic hydrolysis alone is not sufficient to hydrolyze cellulose to hexose sugar; and 2) EH also needs the pre-

ceding degradation of the starch fraction to take advantage of the sugars derived from it. The lower pentose yield (3–4%) also implies that the enzyme action is not sufficient to hydrolyze hemicellulose, and it is necessary to develop a pretreatment that facilitates access to the hemicellulose, and to improve the enzymatic hydrolysis. This result is in accordance with Saha and Bothast (1999).

Hot Water Treatment Followed by Enzymatic Hydrolysis

Hot water treatment enhanced the pentose yield from 3.3 to 9.9%, from 3.8 to 15%, and from 4 to 10.4% of the theoretical yield for enzymatic hydrolysis times of 48, 60, and 72 hr, respectively (Table IV). The hexose yield increased from 31 to 54.6%, from 23 to 71%, and from 12.3 to 39% of the theoretical yield for the enzymatic hydrolysis times of 48, 60, and 72 hr, respectively. Total yield of sugars increased from 11 to 27%, from 8 to 36%, and from 6 to 21% of the theoretical yield for enzymatic hydrolysis times of 48, 60, and 72 hr, respectively. Hot water treatment seems to be an effective method to improve hexose and pentose yields and to facilitate enzymatic hydrolysis. Maximum hexose yield was 71% for 60 hr of EH; however, this percentage is calculated only on the basis of sugars recovered from cellulose because the used enzymes cannot degrade starch. Therefore, the hexose yield was lower than the yield obtained after starch degradation, which suggests that SD is necessary before HW and EH to take advantage of the sugars degraded from the starch. In addition, the weakness of HW-EH impairs the hydrolysis of hemicellulose and the maximum yield was only 15% of the theoretical pentose yield. It is necessary to look for other treatment combinations to improve hemicellulose hydrolysis.

Starch Degradation, Hot Water, and Acid Hydrolysis

After SD, H_2SO_4 was added before HW treatment. Table II shows the chemical composition of bran before and after SD-HW-AH. Treatment with SD-HW-AH degraded $\approx 98\%$ of hemicellulose, decreased starch concentration by 93%, and increased exposure of cellulose to 70%. But pentose yield was only 35% of the theoretical yield (Table IV). During the heat treatment of carbohydrates, appreciable amounts of sugar degradation products or toxic compounds may be formed, depending on the severity of the treatment (Larsen et al 1999). The higher percentage of degradation in hemicellulose and the lower pentose yield suggest that this pretreatment combination could decompose pentose into other compounds. Recovery of total hexose yield was also the lowest among all the treatments (63%). The hexose sugar yield includes sugar yield from SD, which accounts for 60% of the total hexose yield; thus, the recovery of sugars from cellulose was almost null. Total sugar yield was 54% of the theoretical yield, slightly higher than the yield obtained with HW-EH.

Starch Degradation, Hot Water, and Enzymatic Hydrolysis

The combination of starch degradation (to remove starch), HW treatment (to expose and concentrate the remaining cellulose and hemicellulose), and optimization of enzymatic hydrolysis was done as a final attempt to increase the total sugar yield.

TABLE II

Chemical Composition of Original Bran Compared with Composition of Bran After Several Treatments^{a,b}

Component (%)	Sorghum	HW	SD	SD-HW-AH	SD-HW-EH
Starch	29.7	12.3	5.0	1.9	3.5
Hemicellulose	17.5	21.8	22.3	0.2	12.8
Cellulose	10.9	21.3	18.8	36.0	23.3
Acid detergent lignin	0.7	1.1	1.6	2.5	2.3
Total (g)	100	78.0	60.0	24.0	20.0

^a HW, hot water (130°C, 20 min); SD, starch degradation; AH, acid hydrolysis (3%, v/v, H_2SO_4); EH, enzymatic hydrolysis (60 hr, 50°C, and 0.33%, v/v, of each enzyme).

^b Mean values of two replicates.

TABLE III

Yield (%) of Sugars from Cellulose and Hemicellulose After EH and HW-EH Process at Different Times^{a,b}

Sugars	Hydrolysis Time (hr)	EH	HW-EH
Pentoses	48	3.3	9.9
	60	3.8	15.0
	72	4.0	10.4
Hexoses	48	31.0	54.6
	60	23.0	71.0
	72	12.3	39.0
Total	48	11.0	27.0
	60	8.0	36.0
	72	6.0	21.0

^a EH, enzymatic hydrolysis (50°C, 0.33%, v/v, of each enzyme, and pH 5.0); HW, hot water (130°C, 20 min); and EH, enzymatic hydrolysis (50°C, 0.33%, v/v, of each enzyme).

^b Mean values of two replicates.

TABLE IV

Yield (%) of Sugars After Enzymatic Hydrolysis Compared with HW-EH Process, SD-HW-AH Process, and Optimum Response of SD-HW-EH Process^{a,b}

Sugars	EH	HW-EH	SD-HW-AH	SD-HW-EH
Pentoses	3	15	35	63
Hexoses	23	71	63	79
Total	8	36	54	75

^a EH, enzymatic hydrolysis (60 hr, 50°C, and 0.33%, v/v, of each enzyme); HW, hot water (130°C, 20 min); SD, starch degradation; AH, acid hydrolysis (3%, v/v, H_2SO_4).

^b Mean values of two replicates.

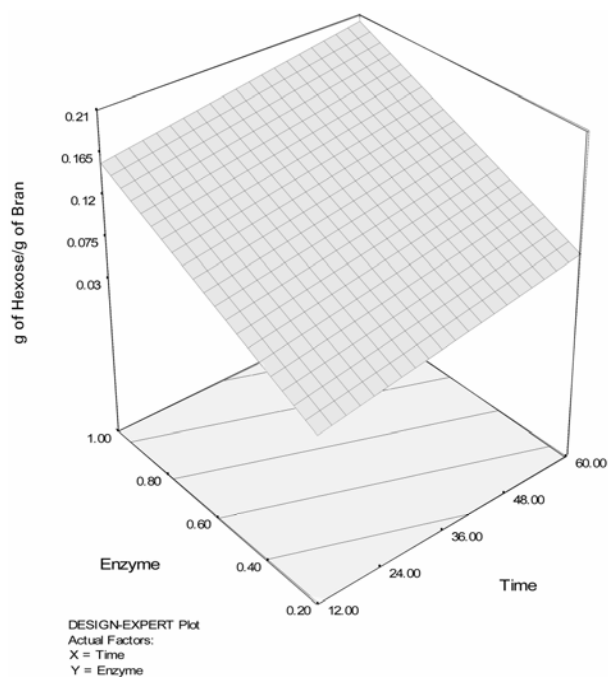


Fig. 3. Response surface of hexose yield after starch degradation, hot water, and enzymatic hydrolysis (SD-HW-EH) treatments.

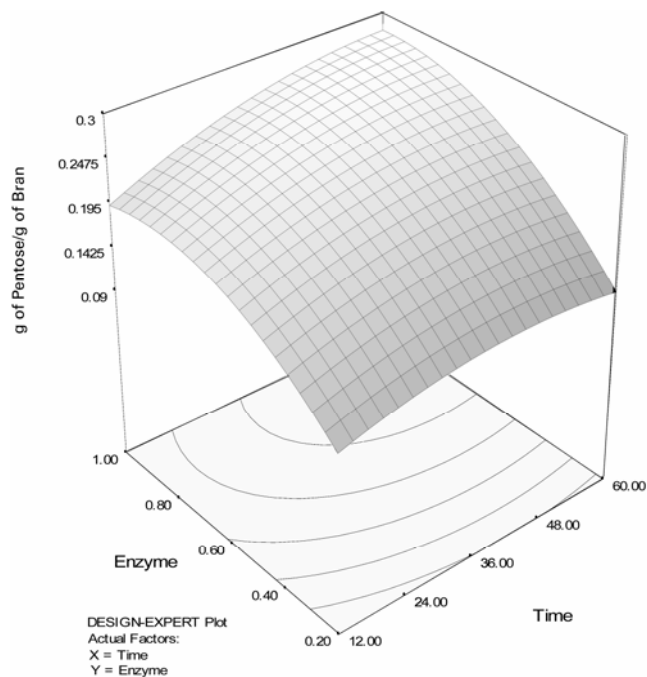


Fig. 4. Response surface of pentose yield after starch degradation, hot water, and enzymatic hydrolysis (SD-HW-EH) treatments.

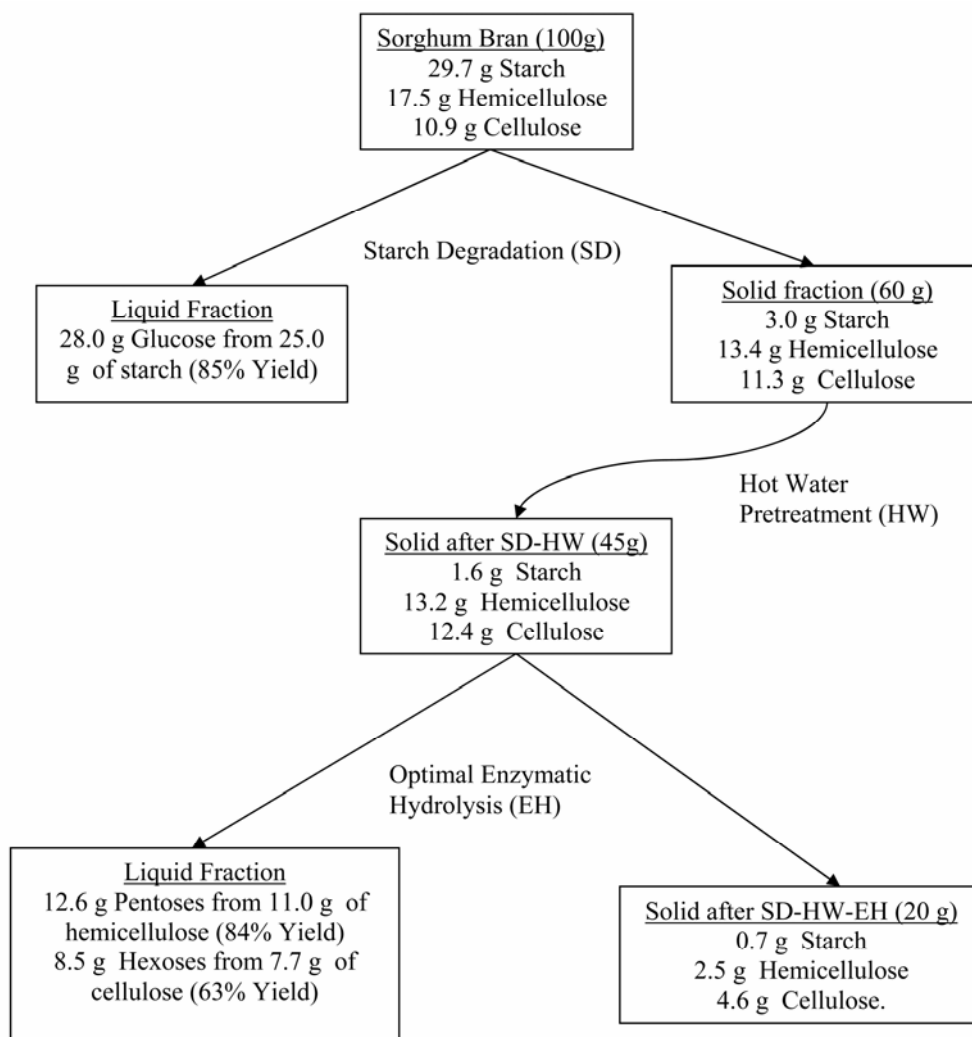


Fig. 5. Mass balance of starch degradation (SD), hot water (HW), and enzymatic hydrolysis (EH) process for cellulose, hemicellulose, and starch components.

Results of treatment were analyzed using RSM. The yield of pentoses and hexoses after treatment was used to calculate the efficiency of the treatment. Linear and quadratic models were developed to study the effects of time and enzyme loads on both hexose and pentose yields.

Figures 3 and 4 show three-dimensional displays of the response surface for hexose and pentose yields after SD-HW-EH treatment, respectively. Maximum hexose yield from the cellulose fraction was 72.5% of the theoretical yield. It was reached at 36 hr with an enzyme load of 1.16%, v/v, per enzyme; the maximum pentose yield (81.8% of theoretical) was reached at 60 hr, with 1%, v/v, of each enzyme. The desirability function was used to maximize the combination of the two responses. The optimal condition was found at 60 hr (0.9%, v/v, of each enzyme) with a desirability function of 0.76. With these conditions, maximum hexose yield from starch and cellulose fraction reached as much as 79% of the theoretical yield (Table IV). Maximum pentose yield reached as much as 63% of the theoretical yield. If SD-HW-EH is compared with EH at 60 hr, the combined process increased pentose yield from 3 to 63%, hexose yield from 23 to 79%, and total sugar yield from 8 to 75% of the theoretical yield. Figure 5 shows the mass balance of the SD-HW-EH process. It illustrates that $\approx 61\%$ of initial hemicellulose and 62% of initial cellulose were converted to sugars.

CONCLUSIONS

Sorghum bran consisting of $\approx 60\%$ carbohydrates from starch, cellulose, and hemicellulose (on a dry basis) proves that it could be an appropriate sugar source for ethanol and other chemical production because of its high carbohydrate content. Carbohydrates from sorghum bran are resistant to enzymatic hydrolysis, probably due to factors such as cellulose crystallinity, surface accessibility, ratio of hemicellulose to cellulose, and less impact from lignin content. Hot water treatment was powerful in concentrating and exposing hemicellulose and cellulose fraction and was optimal when employed at 130°C for 20 min. A method combining SD, HW, and EH was developed to obtain a maximum total sugar yield of 75%. This method consisted of SD, followed with HW treatment at 130°C for 20 min, and EH with 0.9%, v/v, of each enzyme for 60 hr at 50°C. This process improved hexose yield from 23 to 79%, pentose yield from 3 to 63%, and total sugar yield from 9 to 75%.

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